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HTLV-Is in Argentina Are Phylogenetically Similar to Those of Other South American Countries, but Different From HTLV-Is in Africa

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To understand the origin and past dissemination of human T-cell leukemia/lymphotropic virus type I (HTLV-I) in Latin America, we conducted a phylogenetic study of five new HTLV-I isolates from Argentina. We sequenced partial fragments of long terminal repeats (LTR) of the new HTLV-Is, and then the sequences were subjected to a phylogenetic analysis for comparison with other HTLV-Is of various geographical origins. Our results indicated that all the isolates were members of the Cosmopolitan group. Furthermore, most (four out of five isolates) of the new HTLV-Is belonged to the Transcontinental (A) subgroup, the most widespread subgroup of the four subgroups in the Cosmopolitan group. In this subgroup, they were closely related to HTLV-Is found in other South American countries including those of Amerindians, and were different from those found in Africa. In contrast, the remaining one HTLV-I (ARGMF) did not show any clear similarity to known HTLV-I isolates belonging to the Cosmopolitan group. The close similarity of South American HTLV-Is strongly suggests a common origin of the virus in this continent. Our results do not support the proposed idea of recent introduction of HTLV-I into South America as a consequence of the slave trade from Africa, where phylogenetically different HTLV-Is predominate. ***J. Med. Virol. 55: 152–160, 1998.***

KEY WORDS: phylogeny; LTR; South America

INTRODUCTION

South America is now considered to be an endemic area for human T-cell leukemia/lymphotropic virus type I (HTLV-I) [Poiesz et al., 1980; Hinuma et al., 1981], which is the etiological agent of adult T-cell leukemia (ATL) [Uchiyama et al., 1977] and HTLV-I-associated myelopathy/tropical spastic paraparesis (HAM/TSP) [Gessain et al., 1985; Osame et al., 1986]. This virus is also endemic in Japan, Africa and the Caribbean basin [Tajima et al., 1992]. Cases of HTLV-I infection have been found in almost all South American countries including Brazil, Colombia, Argentina, Peru, French Guyana, and Chile [Tajima et al., 1992]. In these countries, HTLV-I has been found in various ethnic populations, including whites, blacks, mestizos, mulattoes and Japanese immigrants. It should be noted that the presence of HTLV-I has also been identified among native inhabitants of South America (Amerindians) in Argentina [Bouzas et al., 1994], Colombia [Duenas-Barajas et al., 1992; Zaninovic et al., 1994], Chile [Cartier et al., 1993; Fujiyoshi et al., 1995], and Brazil [Ishak et al., 1995].

Extensive genetic analyses of various HTLV-I isolates of diverse geographic origins have shown remarkably low genetic heterogeneity, which is related to the geographic distribution and ethnic backgrounds of the virus-carriers rather than to the diverse manifestations of the disease [Gessain et al., 1992; Miura et al., 1994]. The current phylogenetic tree of HTLV-I contains three major lineages: the Cosmopolitan group, the Central African group and the Melanesian group [Franchini, 1995; Gessain et al., 1996; Yamashita et al., 1996; Yanagihara, 1994]. Moreover, recent studies, mostly based on the LTR region, have shown that the Cosmopolitan group is further subdivided into four minor subgroups [Gasmi et al., 1994; Miura et al., 1994; Ureta Vidal et al., 1994]. Indeed, several nucleotide substitutions specific to each subgroup have been observed not only in the LTR sequences but also in the

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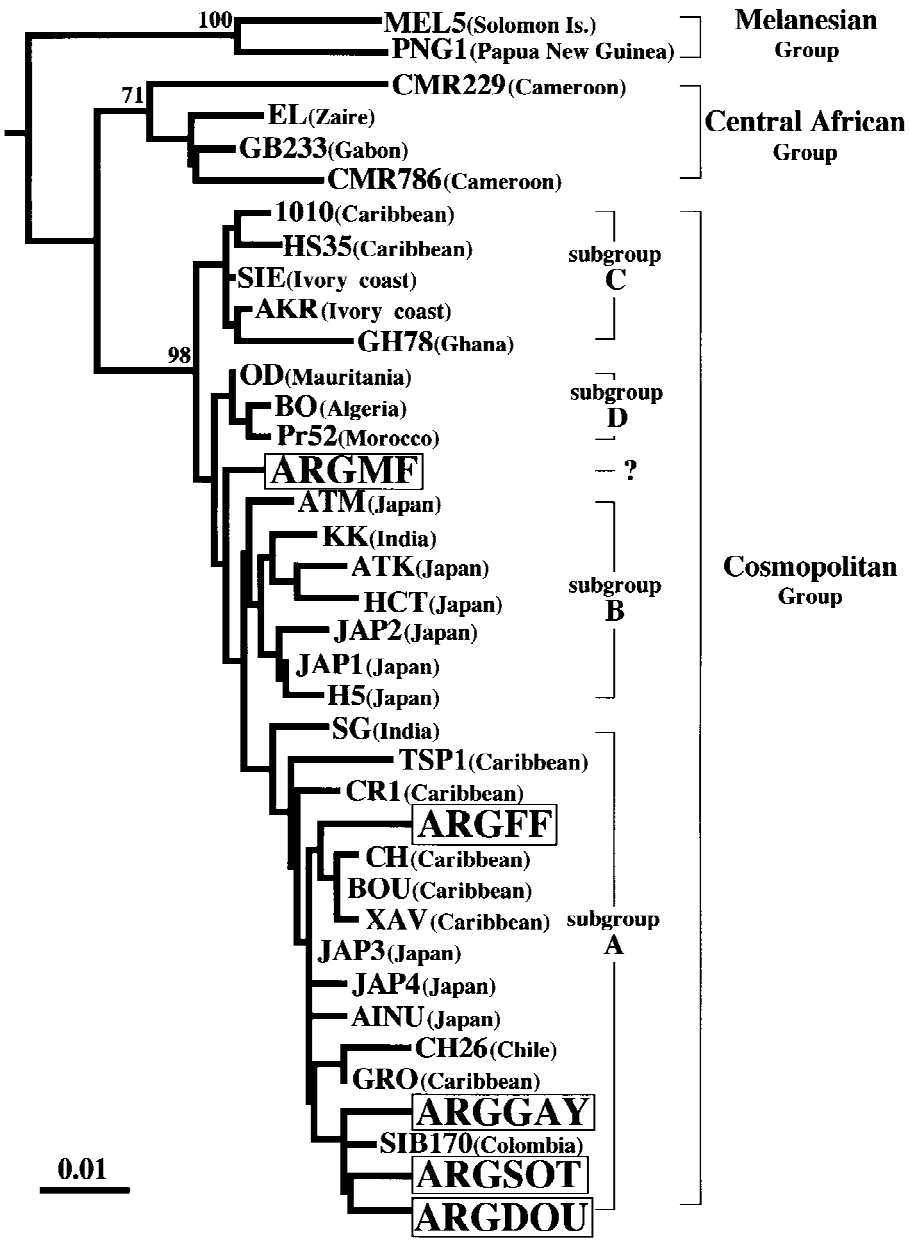

Fig. 1. Phylogenetic tree of HTLV-Is based on parts of the LTR region (nucleotide positions 144–650 in ATK) showing evolutionary relationships between new Argentinean isolates and those previously reported. The scale at the bottom of the tree indicates the number of nucleotide substitutions per site estimated by Kimura's 2 parameter method. The horizontal branch lengths are proportional to the genetic distance, whereas the vertical branch lengths have no significance. The numbers at nodes are bootstrap values. The tree was rooted with a prototype isolate of HTLV-II, MoT [Shimotohno et al., 1985]. All the other DNA sequences used for construction of the phylogenetic tree have been published previously [Dasgupta et al., 1992; Evangelista et al., 1990; Fukasawa et al., 1987; Gasmi et al., 1994; Gessain et al., 1993; Hashimoto et al., 1993; Josephs et al., 1984; Komurian et al., 1991; Malik et al., 1988; Miura et al., 1994; Ono et al., 1994; Ratner et al., 1991; Saksena et al., 1992; Seiki et al., 1983; Seiki et al., 1982; Shirabe et al., 1990; Tsujimoto et al., 1988].

env gene [Lin et al., 1996] and the *tax* gene [Mahieux et al., 1995]. We tentatively designated these four subgroups as the Transcontinental (A), Japanese (B), West African (C) and North African (D) subgroups [Yamashita et al., 1996]. HTLV-I isolates belonging to the Transcontinental subgroup are widely distributed over the world, whereas the other three subgroups have been found in more restricted areas. The Japanese, West African and North African subgroups are limited to their respective areas, except for two isolates of the West African subgroup which were found in the Caribbean basin.

Despite the fact that much effort has been devoted to the genetic analysis of HTLV-I, little is known about which subgroups of the Cosmopolitan group prevail in South America. In fact, a great deal of knowledge about the molecular epidemiology of this virus comes from studies of viral isolates from the main HTLV-I endemic

TABLE I. Deduced RFLP Patterns[a] of LTRs of Argentinean HTLV-I

Isolates	*Nde*I	*Dra*I	*Sac*I	*Mae*III	Proposed subgroup[b]
ARGDOU	+	–	+	–	Cosmopolitan a[c] or e[d]
ARGFF	+	–	+	–	Cosmopolitan a or e
ARGGAY	+	–	+	–	Cosmopolitan a or e
ARGMF	+	–	+	+	African c
ARGSOT	+	–	+	–	Cosmopolitan a or e

[a]RFLP pattern of each isolate was deduced from its nucleotide sequence.
[b]According to the classification proposed by Ureta Vidal et al.
[c]Corresponding to the "Transcontinental" subgroup in our designation.
[d]Since we sequenced partial LTR fragments, no information was obtained that distinguish between Cosmopolitan a and e.

foci, Japan, the Caribbean basin and Africa as well as two recently identified foci, Melanesia and Iran. Therefore, to better understand the genomic features of HTLV-I in South America, we conducted a phylogenetic study of five new HTLV-I isolates from Argentina.

MATERIALS AND METHODS

Subjects

Peripheral blood mononuclear cells (PBMC) were obtained from 5 male HTLV-I seropositive individuals as determined by particle agglutination (Serodia) and Western blot (Diagnostic Biotechnology 2.3). Two patients suffered of ATL (ARGDOU and ARGMF), one was an haemophiliac (ARGGAY) and two were asymptomatic carriers (ARGFF and ARGSOT). Patient ARGDOU lived most of his life in the province of Entre Rios and was 42 years old at the time of his death. ARGMF spent most of his life in Chile and was 54 years old at the time of his death. ARGGAY suffered from severe hemophilia A. During his lifetime he received whole blood transfusions as well as crioprecipitates and commercial clotting factors. ARGFF (51 years) lived in the province of Misiones. ARGSOT (40 years) was from Buenos Aires city. He received massive blood transfusions in 1972 and 1976. With exception of ARGSOT (mestizo), all patients were ethnically white and descendants of European immigrants. DNA was extracted from either cultured or uncultured PBMC by a conventional method using proteinase K.

Polymerase Chain Reaction (PCR)

We amplified approximately 590-bp fragments of the LTR region which correspond to positions 99 to 685 in ATK, the prototype Japanese HTLV-I strain [Seiki et al., 1983]. The nested PCR conditions and the oligonucleotide primers for amplification of the LTR fragments were described previously [Yamashita et al., 1995]. In addition, we amplified a partial *env* fragment of ARGMF which includes the carboxyl terminus of gp46 and almost the entire transmembrane protein gp21 as mentioned previously [Mboudjeka et al., 1997]. Special care was taken in the PCR experiment to avoid contaminating the amplified products. All the genomic DNAs were manipulated in a room free from the amplified products, and a negative control was used in every PCR experiment.

Subcloning and Sequencing

The amplified fragments were cloned into the *Sma*I site of pUC119. Approximately 500 bp-long sequences (positions 122 to 628 in ATK) of the LTR region were determined from the cloned PCR products. In addition, a 522-bp *env* fragment of ARGMF corresponding to positions 6046–6567 in ATK was sequenced. The nucleotide sequences were determined in both directions by using an automated DNA sequencer (Applied Biosystems, Foster City, CA). In general, we sequenced one clone for each sample, since nucleotide sequences from different clones of a sample were virtually identical. However, we sequenced two to three clones for the ambiguous samples. The new nucleotide sequences in the present study have been deposited in GenBank with the accession numbers AF007751–AF007756.

Construction of Phylogenetic Trees

Each set of nucleotide sequences newly obtained and previously reported was aligned by using the computer software CLUSTAL W [Thompson et al., 1994] with minor manual modifications. Phylogenetic trees were constructed by using the neighbor-joining (N-J) [Saitou et al., 1987], and maximum parsimony (MP) methods. For construction of N-J trees, bootstrapping [Felsenstein, 1985] was done to generate 100 resamplings of the original sequence alignments and pairwise genetic distances were estimated on each resampling by Kimura's two-parameter method [Kimura, 1980]. Then, phylogenetic trees were constructed with CLUSTAL W and the trees were visualized by using TREEVIEW [Page, 1996]. For construction of MP trees, we used the phylogeny inference package PHYLIP version 3.52 [Felsenstein, 1993]. One hundred resamplings of the original alignment were generated using the SEQBOOT program. The most parsimonious trees were generated from bootstrapped sequence data using the DNAPARS program. The majority-rule consensus tree was generated using the CONSENSE program. The phylogenetic trees constructed by the N-J method are shown in this report since those by the MP method were virtually identical to those constructed by the N-J method.

RESULTS

We successfully amplified proviral LTR sequences of five new HTLV-I isolates from Argentina. All the new sequences were closely similar to each other, differing

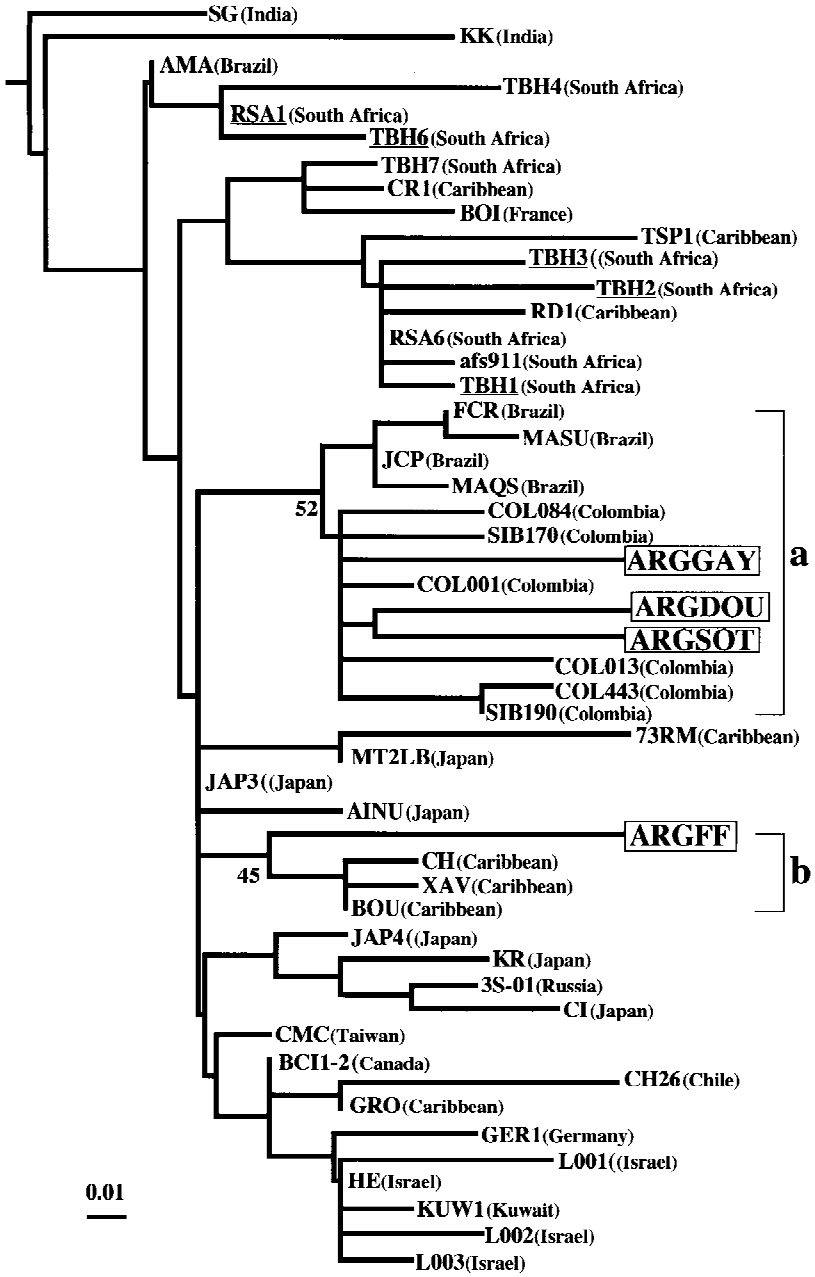

Fig. 2. Phylogenetic tree of HTLV-I isolates that belong to the subgroup A. The tree was constructed based on parts of the LTR region (nucleotide positions 144–650 in ATK). PNG1, one of the most divergent strain of HTLV-I [Saksena et al., 1992], was used to root the tree. Four new Argentinean HTLV-Is are shown in boxes. HTLV-I isolate from South Africa are shown with underline. All the DNA sequences used for comparison have been reported previously, and the detailed information are available in the references shown in the legend to Fig. 1 as well as the following references [Bazarbachi et al., 1995; Chen et al., 1995; Chou et al., 1995; Engelbrecht et al., 1996; Liu et al., 1996; Mahieux et al., 1997; Miura et al., 1997; Picard et al., 1995; Vandamme et al., 1994; Voevodin et al., 1995; Yamashita et al., 1995; Yamashita et al., 1995a; Yamashita et al., 1995b; Yamashita et al., 1995c].

by less than 3.2% among them. A phylogenetic tree of various HTLV-I isolates of diverse geographical origins as well as the five new isolates is shown in Fig. 1. All the Argentinean HTLV-Is were members of the Cosmopolitan group as shown in Fig. 1. Within this group, four out of the five isolates (ARGGAY, ARGSOT, ARGFF, and ARGDOU) belonged to subgroup A. This was confirmed by their RFLP profiles, which were consistent with those of subgroup A in our classification (Table I). Our subgroup A corresponds to the "cosmopolitan" subgroup of Ureta Vidal et al. [Ureta Vidal et al., 1994]. In this subgroup, three of the isolates (ARGGAY, ARGDOU and ARGSOT) clustered with HTLV-Is of both Amerindians and some people of mixed races in Colombia as well as with some HTLV-I isolates from Brazil (Fig. 2). On the other hand, the other isolate (ARGFF) made a cluster with HTLV-I isolates of the Caribbean basin. Some HTLV-I isolates

TABLE II. Specific Sequence Variations of Four Argentinean HTLV-Is

	365[a]	479	528
Consensus[b]	C	T	A
cluster "a"[c]	C	**C**	**G**
ARGDOU	C	**C**	**G**
ARGGAY	C	**C**	**G**
ARGSOT	C	**C**	**G**
cluster "b"[c]	**T**	T	A
ARGFF	**T**	T	A

[a]Nucleotide position is corresponding to that of the prototypic strain, ATK.
[b]All the isolates of the Cosmopolitan group, except for those mentioned here, share the same nucleotide at each position.
[c]HTLV-I isolates belonging to each cluster are shown in Fig. 2.

from Amerindians in Chile belonged to this cluster (unpublished results) as shown in Fig. 2. Although the bootstrap values supporting these two clusters ("a" and "b" in Fig. 2) were not very high (52% for a and 45% for b), we were able to observe specific nucleotide substitutions for each cluster (Table II). All the isolates within cluster "a" have a C at position 479 and G at position 528 in the ATK strain, while all the other isolates of the Cosmopolitan group have a T at position 479 and an A at position 528. In a similar manner, ARGFF had a T at position 365 in common with other HTLV-Is within cluster "b," while in the rest of the Cosmopolitan isolates the nucleotide at position 365 was C. It should be noted that subgroup A has been found only rarely on the African continent except in South Africa. HTLV-I isolates from this country differed from the new Argentinean HTLV-Is as shown in Fig. 2.

As shown in Fig. 1, ARGMF did not belong to any known subgroups in the Cosmopolitan group. It is of interest that the predicted RFLP profile of ARGMF was consistent with that of the African c subgroup proposed by Ureta Vidal et al. [Ureta Vidal et al., 1994] (Table I). In their analysis, only one in 180 samples belonged to the African c subgroup. To better understand ARGMF, we sequenced a 522 bp-long *env* fragment and constructed a phylogenetic tree. Although a phylogenetic tree based on the *env* fragment is not capable of dividing the Cosmopolitan group into four subgroups, large numbers of *env* sequences of various locations are available. Thus, it is possible to determine whether there exist any HTLV-Is that would be completely identical to ARGMF. A phylogenetic tree based on the *env* region indicated that ARGMF was a member of the Cosmopolitan group of HTLV-I, as it was in the LTR-based tree, yet the tree did not show any isolate that is significantly similar to ARGMF (Fig. 3). Recently, Mahieux et al. reported 58 new *env* sequences of African HTLV-1 strains [Mahieux et al., 1997]. Comparison of the 58 *env* sequences with ARGMF also did not find any African HTLV-I strain that closely resembles the Argentinean isolate (data not shown). Taken together, these results suggest that ARGMF is a unique HTLV-I isolate among various isolates of the Cosmopolitan group.

DISCUSSION

This study is the first to phylogenetically characterize HTLV-I isolates from Argentina. HTLV-I is transmitted mainly in a cell-associated manner so that the efficiency of the viral transmission is low. This probably reflects their natural transmission mode, which requires close and frequent contacts, such as breast feedings and sexual contacts. In addition, it appears that blood transfusion and needle sharing among intravenous drug users also contribute to the spread of this retrovirus. Thus, the dissemination of the virus is associated with the movement of HTLV-I carriers as well as the physical contacts between HTLV-I carriers and non-carriers.

The majority of the population in Argentina have European ancestors. The other populations are Amerindians and Mestizos, the latter being of mixed Amerindian and European ancestry. HTLV-I in Argentina is found among European descendants and Mestizos [Bouzas et al., 1990; Bouzas et al., 1994; Muchinik et al., 1993]. In addition, a small number of Amerindians in Argentina have been reported to be infected with HTLV-I [Bouzas et al., 1994]. Considering that Europe is not an endemic focus for HTLV-I [Network, 1996], it is unlikely that ancestors of HTLV-I carriers in Argentina had acquired the virus in Europe and brought it to South America. Rather, it seems plausible to consider that HTLV-I carriers in Argentina acquired the virus in South America.

The present study revealed that four out of five new Argentinean HTLV-I isolates belonged to subgroup A of the Cosmopolitan group. So far, all the HTLV-I isolates in South American countries such as Colombia, Chile and Brazil [Liu et al., 1996; Miura et al., 1994; Miura et al., 1997] have been shown to belong to subgroup A. Thus, this finding implies the existence of a common source of this virus in South America. In consequence, it could be speculated that most Argentinean HTLV-Is have been introduced into its territory from other South American countries. Alternatively, some Amerindian populations might play a key role in the current presence of HTLV-I in this country since the new isolates from Argentina exhibited close similarities to those of Amerindians from Colombia and Chile.

In spite of the apparent association of South American HTLV-Is with subgroup A, it is difficult to speculate about the origin of the unique isolate found in this study (ARGMF). Although the patient infected with ARGMF had lived in Chile for most of his life, the isolate did not show an obvious similarity to any HTLV-Is including Chilean isolates. Thus, at present, there is no clear evidence that ARGMF originated from Chile. Based on its RFLP pattern, similar to the one observed in the African c subgroup, it could be speculated that this isolate has an African origin. Regardless of the true origin of the infecting isolate, it is highly likely that the patient acquired the virus in South America, as it was determined that he had never visited the

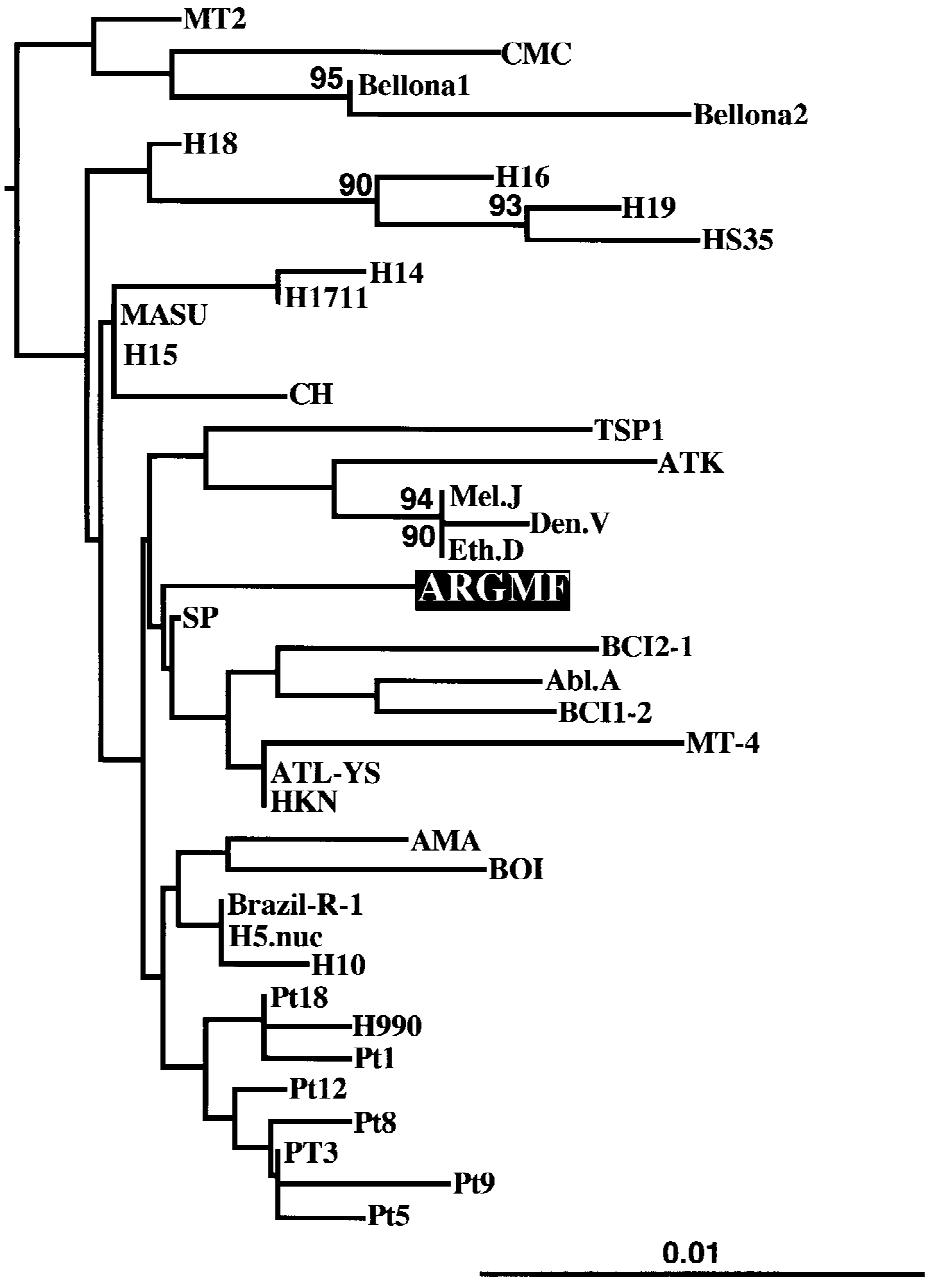

Fig. 3. Phylogenetic tree of 522-bp-long *env* segments of HTLV-Is belonging to the Cosmopolitan group. The new isolate from Argentina (ARGMF) is shown in a box. The tree was rooted with MEL5 [Gessain et al., 1993], which is one of the most divergent strains of HTLV-I and which belongs to the Melanesian group. All the other DNA sequences used for construction of the phylogenetic tree have been published previously [Bazarbachi et al., 1995; Chou et al., 1995; Evangelista et al., 1990; Gessain et al., 1991; Gray et al., 1990; Koralnik et al., 1994; Liu et al., 1996; Mahieux et al., 1994; Malik et al., 1988; Nerurkar et al., 1994; Picard et al., 1995; Ratner et al., 1991; Seiki et al., 1983; Shulz et al., 1991; Tsujimoto et al., 1986]. Nucleotide sequences belonging to the Cosmopolitan group were used in the present study. For details, see the legend to Fig. 1.

African continent and only visited Europe without engaging in high risk practices.

The present results coupled with the previous findings have led us to conclude that the majority of HTLV-Is distributed in South America are closely related to those of Amerindians, but not to those of Japan and Africa. Previously, we suggested that some Amerindian populations have been infected with HTLV-I since their migration into South America approximately 10,000 years ago [Miura et al., 1994; Miura et al., 1997; Yamashita et al., 1996]. This suggestion was based on the fact that these populations have been ethnically isolated. Hence, recent introductions from other populations seem unlikely considering that the efficiency of transmission of this virus is considerably low. Our suggestion has been supported by the presence of this virus among different native inhabitants in North and South America [Fujiyoshi et al., 1995; Kaplan et al.,

1993; Picard et al., 1995]. Furthermore, we speculated that the Mongoloid populations who migrated via the land bridge of Beringia about 10,000 years ago brought HTLV-I from Asia into the New World, resulting in the current presence of subgroup A in Japan and South America, both of which are inhabited by descendants of the Mongoloid population [Miura et al., 1994; Miura et al., 1997; Yamashita et al., 1996; Yamashita et al., 1995b]. In contrast, most researchers have speculated that HTLV-I was spread to South America by African blacks who had been brought as slaves from the 16th to the 19th centuries [Gessain et al., 1992; Mahieux et al., 1997; Song et al., 1995]. However, the previous and present observations do not seem to support this speculation since HTLV-I isolates distributed in Africa are distinct from those in South America. We think the inconsistency between our view and the prevailing view is due to differences in the genomic regions used in the phylogenetic analyses. In contrast to our analysis based on LTR, those who proposed an African origin of HTLV-I in South America have generally used parts of open reading frames (*env, gag* and *pol* genes) [Gessain et al., 1992; Mahieux et al., 1997; Song et al., 1995]. For instance, many HTLV-Is have been phylogenetically characterized based on 522-bp *env* fragments which include the carboxyl terminus of gp46 and almost the entire transmembrane protein gp21 [Gessain et al., 1992; Mahieux et al., 1997]. Comparison of the 522-bp sequences seems suitable for investigating the relationships among heterogeneous strains (i.e. Central African vs. Melanesian vs. Cosmopolitan groups) [Koralnik et al., 1994; Mahieux et al., 1997]. However, the comparison seems inadequate when examining the differences among more homogeneous viral isolates such as among HTLV-I isolates that belong to the Cosmopolitan group, because the analysis of the 522-bp sequences does not differentiate the four subgroups of the Cosmopolitan group. In fact, Lin et al. have indicated that most subgroup-specific nucleotide variations in the *env* gene are found within the gp46 encoding region and only one variation is observed within the 522-bp sequences [Lin et al., 1996]. It is likely that the absence of such subgroup-specific variations in the 522-bp sequences resulted in their finding that all the isolates of the Cosmopolitan group, including some isolates of Africa as well as South America, are very similar to each other. However, as shown by us and others, phylogenetic analyses based on the LTR region have indicated that HTLV-I strains which prevail in Africa are not prevalent in South America [Miura et al., 1994; Miura et al., 1997; Ureta Vidal et al., 1994]. Therefore, at present, we consider that most HTLV-Is in South America have not originated from Africans brought during the period of the slave trade. Nonetheless, further analyses are needed because genetic analyses which can divide the Cosmopolitan group into four subgroups have not been performed on HTLV-Is from West Africa, the main area from where African blacks were brought to South America as slaves.

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